1 Beyond nonsense mediated RNA decay: exploring functions of UPF1 in translation

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1.1 SUMMARY

UPF1 is an evolutionary conserved helicase essential in most eukaryotes, largely known for its role in nonsense-mediated RNA decay. Interestingly, UPF1 binds co-transcriptionally to a vast portion of mRNAs, not only the ones destined for degradation by NMD. We took advantage of viable UPF1-null mutants in *Arabidopsis thaliana* to determine the consequence of UPF1 inactivation on RNA metabolism. Our data suggest functions of UPF1 far beyond NMD in processes such as splicing, genome stability and translation. Here, we propose to investigate the role of UPF1 in translation and ribosome-associated quality control pathways in collaboration with Winz lab at JGU.

1.2 SCIENTIFIC BACKGROUND

UPF1 is a multifunctional evolutionary conserved ATP-dependent helicase that has been implicated in many cellular processes. The best studied function of UPF1 is in the nonsense-mediated RNA decay (NMD) pathway, which is required to remove aberrant RNAs containing premature termination stop codons (Kim and Maquat, 2019). NMD is coupled to translation and it is believed that NMD target recognition happens probabilistically either during the pioneer or subsequent round of translation. NMD does not only target aberrant transcripts, but a number of studies indicate that it has also acquired important function in post-transcriptional gene regulation (Nasif *et al.*, 2018). As a consequence, NMD has been tightly integrated in regulation of responses to developmental and environmental cues.

UPF1 is the central NMD factor. It binds co-transcriptionally along mRNAs, and during translation it is pushed towards the 3´UTR by the translating ribosome. NMD occurs when a ribosome encounters a premature termination codon (PTC). Termination of translation is different in the context of PTCs than at a normal termination codon, and can lead to phosphorylation of UPF1. This is the defining event of NMD that triggers degradation of such aberrant mRNAs. However, the role of UPF1 in cytoplasmic RNA decay is not only limited to the NMD, as other RNA degradation pathways seem to converge on UPF1 (Kim and Maquat, 2019). In NMD, the function of UPF1 is not restricted only to PTC recognition and mRNA degradation, but it also inhibits re-initiation of the translation of aberrant mRNAs (Isken *et al.*, 2008). In fact, UPF1 has been suggested to maintain translation fidelity and recycle ribosomes from premature translation codons (PTC) (Celik *et al.*, 2015; Celik *et al.*, 2017). It is not clear whether UPF1 rescues ribosomes only from PTCs, or also from other obstacles present on the messenger RNA. An additional role of UPF1 in the ribosome-associated protein quality control pathway has been inferred from the discovery of UPF1´s E3 ubiquitin ligase activity and its ability to facilitate degradation of the nascent peptides derived from the PTC containing transcripts (Kuroha *et al.*, 2009; Feng *et al.*, 2017).

Inactivation of UPF1 results in lethality in a majority of higher eukaryotes (Chang *et al.*, 2007). Therefore, it is difficult to study the consequence of UPF1 on RNA metabolism, especially in the context of the whole organism and its development. We discovered that impairment of NMD in the model plant *Arabidopsis thaliana* leads to a massive activation of pathogen defense (Riehs-Kearnan *et al.*, 2012; Gloggnitzer *et al.*, 2014). With this knowledge, we have overcome the lethality issue by genetic abrogation of the plant immune response. We used the viable NMD mutants in extensive comparative transcriptome and translatome analysis. Interestingly, the inactivation of UPF1 led to translational repression, as manifested by a global shift in mRNAs from polysomes to monosomes and the downregulation of genes involved in translation and ribosome biogenesis (Raxwal *et al.*, 2020). These data hint on a more general role of UPF1 in translation. **In the proposed project, we want to explore this lead and dive deeper into understanding the role of UPF1 in promoting translation and ribosome-associated quality control mechanisms.**

1.3 AIMS

The aims of this project are:

1) To assess the impact of UPF1 inactivation on translation

2) To map the UPF1-RNA interaction sites in Arabidopsis thaliana

1.4 WORK PROGRAM

Aim1. Impact of UPF1 on translation: We will utilize the UPF1-null plants as well as plants generated by complementing UPF1-null mutants with the UPF1 gene carrying the G513R mutation that abolishes its ATPase activity. ATPase deficient UPF1 is unable to dissociate from RNA and accumulates in cytoplasmic ribonucleoprotein particles (RNPs). We will map ribosome binding positions along mRNAs in wild type as well as UPF1 mutants using Ribo-seq. Ribo-seq will determine not only the binding footprint of the ribosome on mRNAs, but also provide information on their translation status. The comparative analysis will show the effect of UPF1 on the rate of translation and recycling of ribosomes from aberrant stalls. We will pay particular attention to transcripts that do not show typical features of NMD substrates. Furthermore, we will investigate whether UPF1 deficiency promotes ribosome collisions which may arise from the failure to stop translation initiation in RNPs containing stalled ribosomes. To undertake this task, we will perform footprinting of colliding ribosomes using Disome-seq. We will also perform RNA-seq with the same material to assess and statistically evaluate enrichment from Ribo-seq and Disome-seq data. Based on comparative data analysis, we will assess whether UPF1 is required to recycle ribosomes and prevent ribosome collision.

Aim 2. Mapping UPF1-RNA interaction sites: In parallel to translatome analysis, we will identify UPF1 binding sites along mRNAs by UV crosslinking and analysis of cDNA (CRAC). For this purpose, we will use plants carrying tagged constructs of UPF1 that complement UPF1-null mutation. We have already produced such *Arabidopsis* lines expressing GFP- and Myc-tagged wild-type as well as ATPase deficient UPF1 proteins. UPF1 RNA biding sites will be correlated with the Ribo-seq data and convergence of these datasets will indicate causality between UPF1 binding and resolution of translation stalls.

1.4.1 What will be done by which partner and the expected synergies

NMD in *Arabidopsis* represents a main area of research in Riha lab and the UPF1 project is carried out by Dr. Vivek Raxwal. He has expertise in plant molecular biology and bioinformatics, and will perform the majority of experiments described in the project. He will be assisted with a PhD student, Surendra Saddala, who will focus on establishing CRAC to study protein-RNA interaction in plants. Dr. Marie-Luise Winz has interests in translation associated quality control and has developed many RNA-protein interaction technologies coupled with high throughput sequencing. She will guide optimization of and provide training in the wet-lab and analysis methods described in this application for plant cells. Her experience in the field of translation quality control will also be invaluable for data interpretation. The project will be carried mainly in years 2022 and 2023.

1.4.2 The benefit of this research project to RNA research at CEITEC

First, through this collaboration we will establish state-of-the-art methods to study protein-RNA interactions in plants and apply these methods to investigate interesting biological questions. Second, we believe that this project can yield high impact discoveries, and eventually good quality publications. Thus, this will increase scientific excellence and reputation of CEITEC in the RNA research community. Third, this project brings together research groups with complementary expertise and working with different model systems, but studying related biological questions. Communication between the laboratories through this project will create synergies and can mutually increase the quality of research outputs of both laboratories.

1.4.3 Future collaborations or joint funding expected to result from work proposed

We perceive this as a pilot project to establish collaboration between two labs that did not collaborate before. Thus, at the moment we do not plan, but do not exclude, for the future, to apply for external funding beyond this INTEG-RNA project.

1.5 BUDGET

The first part of the budget includes standard consumables required to work on Aim 1 and 2. We will require approximately 3800 Euros to purchase these consumables. Some of the required consumables are listed below:

Illumina TruSeq® Stranded Total RNA Library Prep for Plant and their corresponding adapters, Polynucleotide kinase, 4–12% NuPAGE Bis-Tris gel, TriReagent, CircLigase II, Qubit dsDNA HS Assay Kit, GFP Trap beads, cOmplete EDTA-free protease inhibitor, cycloheximide, GlycoBlue, SUPERase Inhibitor, RNase I, T4 RNA ligase, Micro Bio-Spin P30 column, SuperScript IV reverse transcriptase, RNA and DNA oligonucleotides, streptavidin beads (magnetic), T4 RNA ligase 2, RNaseIN, RNace IT, Thermosensitive Alkaline Phosphatase.

We will require to sequence the libraries generated in Aim 1 and Aim 2 on Illumina`s NovaSeq S1 flow cell to get the necessary depth for statistically significant analysis. This will cost us approximately 5200 Euros. The sequencing will be done at Vienna biocentre core facility. Two researchers (Dr. Vivek Raxwal and Surendra Saddala) will visit Dr. Marie-Luise Winz' lab at JGU twice for 2-3 weeks, each. If required, the budget of this project will be supplemented with another running project from the lab.

1.6 REFERENCES

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